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Influence of 5-hydroxytryptamine uptake on the apparent 5-hydroxytryptamine antagonist potency of metoclopramide in the rat isolated superior cervical ganglion

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1 Metoclopramide, 1×10^{-6}–1×10^{-4} M, was found to behave as a reversible, competitive antagonist of 5-hydroxytryptamine (5-HT)-induced depolarization of the rat isolated vagus nerve (VN) and superior cervical ganglion (SCG). The pK_B values were 6.60 (± 0.04) and 5.74 (± 0.07), respectively. The possibility that this apparent difference in potency was due to saturable 5-HT uptake was investigated.

2 The SCG, but not the VN, accumulated tritium-labelled 5-HT via a saturable, sodium- and temperature-dependent mechanism. Ganglionic 5-HT uptake was blocked by desmethylimipramine (IC_{50} 1.4×10^{-6} M), chlorimipramine (8.7×10^{-9} M), zimelidine (1.5×10^{-7} M), paroxetine (4.3×10^{-8} M) and citalopram (6.2×10^{-8} M).

3 The 5-HT uptake inhibitor paroxetine, 1×10^{-6} M, did not modify the apparent 5-HT antagonist potency of metoclopramide on the VN, but raised the pK_B obtained against 5-HT on the SCG from 5.74 (± 0.07) to 6.25 (± 0.03).

4 It is suggested that the observed difference in the potency of metoclopramide as a 5-HT antagonist on the rat VN and SCG was due to saturable 5-HT uptake in the latter preparation. The results do not support a difference in the 5-HT receptors mediating depolarization on the VN and SCG.

Introduction

The potencies of certain antagonists, selective against the actions of 5-hydroxytryptamine (5-HT) on mammalian peripheral neurones, appear to depend upon the preparation used. Such observations support the existence of sub-types of peripheral neuronal 5-HT receptors (see Fozard, 1984; Richardson *et al.*, 1985). 5-HT depolarizes both the rat isolated superior cervical ganglion (SCG) and vagus nerve (VN) (Watson, 1970; Ireland *et al.*, 1982). However, it is not known whether the receptors in these preparations are the same. In order to address this problem, we have compared the actions of metoclopramide against these 5-HT-induced responses on the two tissues.

In a previous study (Ireland *et al.*, 1982), we described how metoclopramide behaves as a reversible competitive antagonist of 5-HT-induced depolarization of the VN for which the negative log of the apparent equilibrium dissociation constant (pK_B) was 6.60 (± 0.04). In contrast, in preliminary studies on the SCG, the pK_B value for metoclopramide was 5.74 (± 0.07). This result may indicate that the 5-HT receptors on the rat VN and SCG are different. However, an alternative explanation comes from the work of Langer & Trendelenburg (1969), who proposed that the potency of an antagonist may be underestimated if the agonist against which it is tested is the substrate for a saturable uptake process.

The existence of an uptake process that specifically accumulates 5-HT has been well documented for both rat brain and blood platelets (see review by Ross, 1982). 5-HT may also be a substrate for noradrenaline uptake processes (Burgen & Iversen, 1965; Shaskan & Snyder, 1970). The rat isolated SCG has been found to accumulate tritium-labelled noradrenaline (Brown & Caulfield, 1979), but the potential of this tissue, or of the rat VN, to accumulate 5-HT has not been des-

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cribed previously. We have examined both tissues for their abilities to accumulate radiolabelled 5-HT and have attempted to characterize any processes involved. We have also sought to quantify the possible influence of 5-HT uptake on the 5-HT antagonist activity of metoclopramide by the use of 5-HT uptake inhibitors, and to compare the results obtained with the quantitative prediction of the model of Furchgott (1972).

A preliminary account of this work has been presented to the British Pharmacological Society (Ireland *et al.*, 1983).

Methods

Preparation of tissues

All experiments were performed on freshly-dissected superior cervical ganglia (SCG), or 10–20 mm lengths of cervical vagus nerve (VN) (minus the nodose ganglion), excised from male Lister-hooded rats (01ac) anaesthetized with chloral hydrate (300 mg kg^{-1} i.p.). Animals weighed between 250 and 350 g.

The connective tissue capsule around each isolated VN or SCG was removed; tissues were maintained in Krebs-Henseleit medium (> 25 ml per tissue) at room temperature (approximately 21°C), gassed with 95% O_2 and 5% CO_2, until use. For uptake experiments only, all nerve trunks were removed from each SCG and the medium contained nialamide, 1×10^{-5} M, unless otherwise stated.

Extracellular recording

5-HT-induced depolarization was recorded extracellularly from VN or SCG preparations mounted in two-compartment perspex baths, as described previously (Ireland *et al.*, 1982). Briefly, each VN was positioned so that 50 to 70% of the nerve lay in the first compartment, while the remainder projected through a greased slot (Dow-Corning high vacuum grease) into the second. Each SCG was mounted with the ganglion lying in the first compartment and the internal carotid nerve containing postganglionic fibres, projecting through the greased slot into the second. The d.c. potential between the two compartments was recorded via silver-silver chloride electrodes connected to the preparation through agar-saline and filter paper bridges and was displayed on a potentiometric chart recorder (Servoscribe 200). Each compartment was perfused continuously at a constant rate of approximately 1 ml min^{-1} with Krebs-Henseleit medium dripped directly onto the tissue. Drugs were applied at known concentrations via the superfusion stream into the first compartment only.

The temperature of each preparation was maintained at 27 ± 1°C by passing solutions through heat exchangers immediately before applying them to the tissue, and by placing the recording bath and electrodes in a temperature-controlled chamber. This temperature was chosen since in preliminary experiments, recorded base-lines were more stable at 27°C than at 37°C (results not shown).

Measurement of monoamine uptake

Individual VN segments or superior cervical ganglia were pre-incubated in 1.9 ml of Krebs-Henseleit medium, maintained at 27°C, and continuously gassed with 95% O_2 and 5% CO_2 or 100% O_2 as appropriate. Generally, compounds to be examined for ability to inhibit uptake were added at the beginning of this period. A pre-incubation time of 60 min was found to be adequate for the equilibration of uptake inhibitors and was therefore used routinely.

Uptake of [^{3}H]-5-HT was studied over a wide range of concentrations (1×10^{-8}–1×10^{-4} M); in some experiments the accumulation of (−)-[^{3}H]-noradrenaline ((−)-[^{3}H]-NA), rather than 5-HT, was examined. For these latter studies, the incubation medium contained ascorbate, 5×10^{-5} M. Incubation was initiated by adding 100 µl of radioligand solution to each tissue preparation. A standard incubation time of 20 min was used routinely, although uptake occurring during both longer and shorter periods was also measured. Uptake was terminated by filtration under vacuum. Tissues were washed with 5 ml of ice-cold Krebs-Henseleit medium and weighed 0.5 and 1.0 min after exposure to room air. Fresh mass was determined by extrapolation to zero time (Brown *et al.*, 1971). Recorded mass ranged from 0.5–1.1 mg for the SCG, 0.6–1.4 mg for the VN. Tissues were dissolved in 0.5 ml of Soluene-350 (Packard). Radioactivity was determined by liquid scintillation spectrometry. Samples were corrected individually for quenching, using the external standard pulse method. Counting efficiencies ranged from 40–50%.

Quantification of uptake inhibition

In experiments in which compounds or manipulations were used to block uptake, results are expressed as the percentage inhibition of the control uptake, measured in separate tissues, in the same experiment.

The potency of each inhibitor was expressed in terms of an IC_{50} value, which was the concentration of the inhibitor calculated to produce 50% of its own maximum effect.

Radiochemical purity

Radiochemical purity was checked by thin-layer chromatography on Avicel plates (Anachem Ltd),

using two solvent systems (ethanol:ethanoic acid: water, 25:4:10 by volume, and *n*-butanol:ethanoic acid:water, 6:3:1 by volume).

In the absence of a monoamine oxidase inhibitor, significant amounts of a total radioactivity recovered from homogenates of ganglia previously incubated with [^{3}H]-5-HT, 1×10^{-8}–1×10^{-5} M for 60 min, co-migrated with 5-hydroxyindoleacetic acid (5-HIAA). This amounted to 31% after exposure to [^{3}H]-5-HT, 1×10^{-8}LH M and 55% after exposure to [^{3}H]-5-HT, 1×10^{-5} M. The conversion of [^{3}H]-5-HT to [^{3}H]-5-HIAA was unlikely to have been an artefact of the preparative procedure. Thus, in control experiments in which ganglia, not previously exposed to [^{3}H]-5-HT, were homogenized in the presence of the radioligand, less than 5% of the total radioactivity co-migrated with 5-HIAA. In ganglia treated with the monoamine oxidase inhibitor nialamide, 1×10^{-5} M, at least 90% of the radioactivity accumulated during incubation with [^{3}H]-5-HT, 1×10^{-8} M for 60 min, co-migrated with 5-HT. After incubation with [^{3}H]-5-HT, 1×10^{-5} M for 60 min, the proportion was 83%. The corresponding estimates for [^{3}H]-5-HIAA were 4% and 13%, respectively. Nialamide, 1×10^{-5} M, was used routinely in all further studies.

Brown & Caulfield (1979) found that 97% of the radioactivity extracted from rat SCG after incubation with (−)-[^{3}H]-NA for 2 h in the presence of nialamide, 1×10^{-5} M, was unchanged amine. This was not checked in the present experiments.

Drugs and solutions

The composition of the normal Krebs-Henseleit medium used in the present study was (in mmol l^{-1}): NaCl 118, $NaHCO_3$ 25, KH_2PO_4 1.18, KCl 4.7, $MgSO_4$ $7H_2O$ 1.18, $CaCl_2$ 2.5, glucose 11. Na^+-free medium was prepared by substitution of LiCl (118 mM) for NaCl (118 mM) and Tris HCl (pH 7.4, 25 mM, Sigma) in place of $NaHCO_3$ (25 mM). This solution was gassed with 100% O_2 rather than 95% O_2 and CO_2. The media were prepared in glass-distilled water and reagents, which were all AR-grade, were obtained from commercial sources.

The drugs used were: 5-HT creatinine sulphate (Sigma), 1-phenylbiguanide (Aldrich), (−)-noradrenaline bitartrate (Sigma), metoclopramide hydrochloride (Beecham), ouabain (strophanthin G, Sigma), desmethylimipramine hydrochloride and chlorimipramine hydrochloride (Ciba), zimelidine dihydrochloride (Astra), citalopram hydrobromide (Lundbeck), paroxetine hydrochloride (Ferrosan), nialamide (Sigma), and 5-hydroxyindoleacetic acid (Sigma).

Tritium-labelled 5-HT creatinine sulphate (15–30 Ci $mmol^{-1}$), and (−)-noradrenaline (47.7 Ci $mmol^{-1}$) were purchased from New England Nuclear.

Curve fitting procedures

The methods used for fitting hyperbolic and logistic curves were based on those of Parker & Waud (1971), which were in turn specializations of the general method of Snedecor & Cochran (1968). The computer programs used for curve fitting in the present study were written by Miss F.J. Illingworth, Department of Computer Science, Glaxo Group Research Ltd, Greenford, Middx.

Results

Antagonism of 5-HT-induced depolarizations of the vagus nerve and superior cervical ganglion in the absence of uptake inhibitors

On the VN, 5-HT, 1×10^{-7}–3×10^{-5} M, caused rapid concentration-dependent depolarization responses with a maximum amplitude for a given tissue of between 300 and 600 μV. Similar depolarization responses were obtained on the SCG, using 5-HT concentrations ranging from 1×10^{-6} to 3×10^{-4} M. On both preparations, repolarization following 5-HT wash-out took 10–20 min; the time-course sometimes appeared biphasic, with a prolonged late component. In the present study, VN and SCG preparations were always allowed to repolarize fully between agonist applications. Agonists were left in contact with the tissues until apparent equilibrium was reached–this usually took 3 min or less. Two sequential control concentration-response curves obtained on the same VN or SCG at 1–2 h intervals were virtually superimposable. Therefore, a standard procedure was used for the measurement of the potency of antagonists. Only one concentration was applied to each VN or SCG; its effects were measured only once it had achieved apparent equilibrium. This was taken to have occurred when repeated application of an approximate EC_{50} of the agonist gave responses equal to within ± 10% of each other. Lateral displacements of concentration-depolarization response curves were measured at the control half-maximal response level. pK_B values were calculated as the mean (± s.e.mean) of the individual results: $pK_B = \log (\text{dose-ratio} - 1) - \log (\text{antagonist concentration})$.

As previously found (Ireland *et al.*, 1982), metoclopramide, 3×10^{-6}–1×10^{-4} M, caused parallel rightward shifts of the 5-HT concentration-response curve on the VN with no significant change in the maximum response (Figure 1). A plot of these data according to the method of Arunlakshana & Schild (1959), and a gradient of 0.98 (± 0.07) (Figure 2). The pK_B value was 6.60 (± 0.04, $n = 16$). On the SCG, metoclopramide, 3×10^{-6}–1×10^{-4} M, also caused parallel rightward displacements of the 5-HT concen-

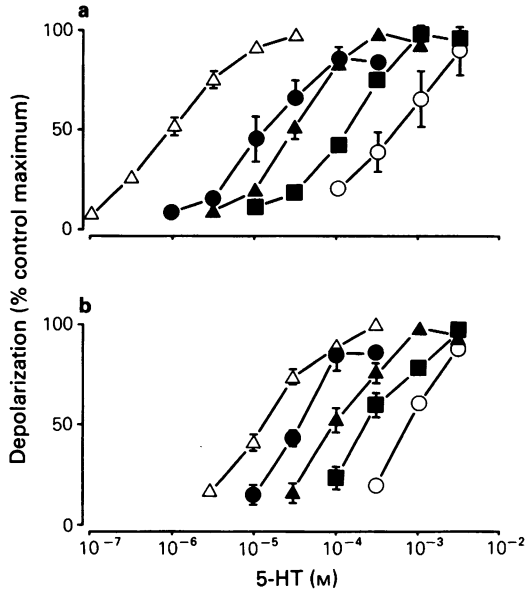

Figure 1 Antagonism by metoclopramide of 5-hydroxytryptamine (5-HT)-induced depolarization of the rat isolated vagus nerve (a) and superior cervical ganglion (b). Symbols indicate controls (△), and the presence of metoclopramide 3×10^{-6} M (●), 1×10^{-5} M (▲), 3×10^{-5} M (■) and 1×10^{-4} M (○). Each point is the mean, with vertical lines indicating s.e.mean, of single determinations from at least 4 separate tissue preparations, each obtained from a different rat.

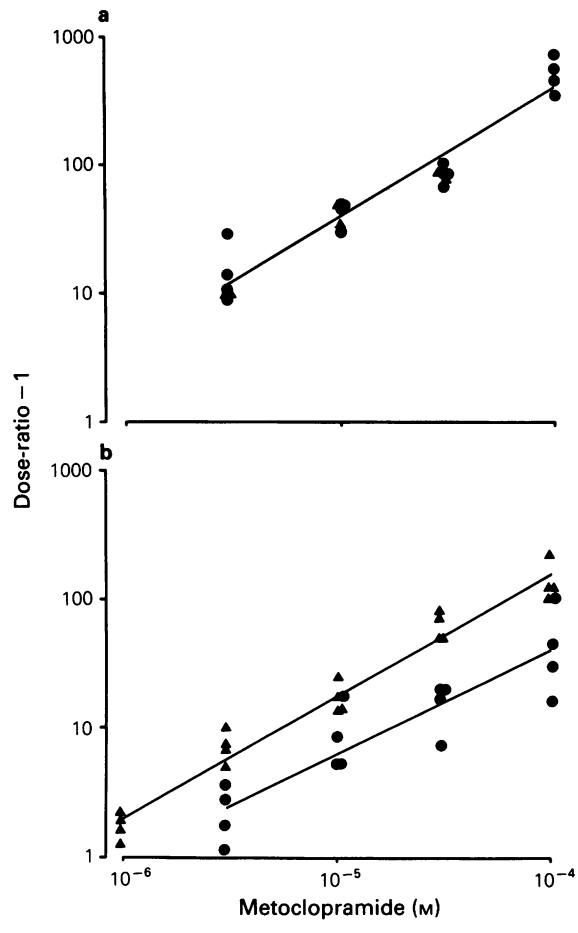

Figure 2 Schild plots of (dose-ratio − 1) against antagonist concentration for metoclopramide antagonism of 5-hydroxytryptamine (5-HT)-induced depolarization of the rat isolated vagus nerve (a) and superior cervical ganglion (b). Each point represents the result obtained on a separate tissue. (●) Data obtained in the absence of a 5-HT uptake inhibitor, (▲) results obtained in the presence of paroxetine, 1×10^{-6} M. Lines were fitted by linear regression.

tration-depolarization curve (Figure 1). However, the Schild plot constructed with these data had a gradient of 0.82 (± 0.12), although this was not significantly different from unity ($P > 0.05$, Students t test) (Figure 2). The pK_B value was 5.74 (± 0.07, $n = 16$).

Accumulation of radiolabelled monoamines by the superior cervical ganglion and vagus nerve

[^{3}H]-5-hydroxytryptamine After incubation with [^{3}H]-5-HT, 1×10^{-8}–1×10^{-5} M, ganglia were found to have accumulated radioactivity against a concentration gradient. The accumulation was linear with time for at least 60 min. The mean tissue-to-medium ratio achieved in ganglia incubated with [^{3}H]-5-HT, 1×10^{-8} M, for 60 min was 26.9 ± 2.4 ($n = 3$). The attained tissue-to-medium ratios were observed to decrease with increased substrate concentration. This effect was significant ($P < 0.001$, analysis of variance on $\log_{10}$ transformed data) at all incubation times from 1 to 60 min. A standard incubation time of 20 min was used subsequently to estimate initial uptake rates.

Vagus nerves incubated with [^{3}H]-5-HT, 1×10^{-8} – 1×10^{-4} M, were also found to accumulate radioactivity. However, the tissue-to-medium ratios achieved were much smaller than those observed in ganglia – the maximum attained in any preparation was 4.1. The rate of accumulation appeared to decrease after 2 min exposure to the radioligand. Tissue-to-medium ratios measured after incubations of 2 min or less did not change significantly with substrate concentration ($P > 0.05$, analysis of variance on $\log_{10}$ transformed data). Therefore, in contrast to the SCG, the VN did not appear to possess a saturable 5-HT uptake system.

Table 1 Effects of various procedures producing inhibition of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake in the rat superior cervical ganglion

	Inhibition of [^{3}H]-5-HT uptake (mean % ± s.e.mean)			
Treatment	*[^{3}H]-5-HT 1 × 10^{-8} M*	(n)	*[^{3}H]-5-HT 1 × 10^{-5} M*	(n)
Cold (4°C)	79.5 ± 1.0	(6)	77.3 ± 1.1	(6)
Ouabain, 1 × 10^{-3} M	61.2 ± 2.2	(5)	56.6 ± 1.7	(6)
Na^+-free Krebs	68.2 ± 2.1	(5)	52.7 ± 3.0	(6)

Tissues were pre-incubated in the test medium for the 60 min immediately before, and during incubation with [^{3}H]-5-HT. In the Na^+-free medium, LiCl (118 mM), and Tris HCl (25 mM, pH 7.4) substituted for NaCl (118 mM) and $NaHCO_3$ (25 mM) respectively.

(−)-[^{3}H]-noradrenaline SCG preparations incubated with (−)-[^{3}H]-NA, 1 × 10^{-8} M, accumulated radioactivity against a concentration gradient. Uptake was linear with time for at least 80 min (result not shown).

Inhibition of ganglionic monoamine uptake

The accumulation of radiolabel by ganglia during incubation with [^{3}H]-5-HT, 1 × 10^{-8} M and 1 × 10^{-5} M, was found to be temperature-sensitive and Na^+-dependent. The uptake was also inhibited by ouabain at 1 × 10^{-3} M (Table 1), but not 1 × 10^{-4} M. This observation accords with the relative insensitivity of the rat SCG to this cardiac glycoside (see Brown & Scholfield, 1974).

The accumulation of radioactivity during incubation with [^{3}H]-5-HT, 1 × 10^{-8} M, was also reduced in ganglia exposed to the uptake inhibitors chlorimipramine, 1 × 10^{-9}–1 × 10^{-4} M, desmethylimipramine, 3 × 10^{-7}–1 × 10^{-4} M, zimelidine, 3 × 10^{-8}–1 × 10^{-4} M, paroxetine 1 × 10^{-8}–1 × 10^{-4} M. and citalopram, 1 × 10^{-8}–1 × 10^{-4} M (Table 2). The accumulation of radioactivity by ganglia during incubation with (−)-[^{3}H]-NA, 1 × 10^{-8} M, was inhibited by desmethylimipramine, 1 × 10^{-9} –3 × 10^{-5} M, and paroxetine, 1 × 10^{-7}–1 × 10^{-4} M.

Ganglionic monoamine uptake was also susceptible to inhibition by agonists. In contrast to the other inhibitors, these were added to the incubation medium concurrently with the radioligand. [^{3}H]-5-HT accumulation was inhibited by 5-HT, 1 × 10^{-8} –1 × 10^{-4} M, but not by phenylbiguanide, 1 × 10^{-8}–1 × 10^{-4} M, or (−)-NA, 1 × 10^{-8} –1 × 10^{-4} M. In contrast, (−)-[^{3}H]-NA uptake was inhibited by both (−)-NA, 1 × 10^{-8}–1 × 10^{-4} M, and 5-HT, 1 × 10^{-8}–1 × 10^{-4} M.

In all the above cases, inhibition was concentration-related. It was found empirically that the logistic curve was a good fit to the experimental data, all deviations

Table 2 Effects of uptake inhibitors on monamine uptake in rat superior cervical ganglia

	Inhibition of 5-HT uptake			*Inhibition of NA uptake*
Compound	*IC_{50}* (μM)	*Maximum* (mean % ± s.e.)	(n)	*IC_{50}* (μM)
Chlorimipramine	0.0087	81.1 ± 0.8	(5)	–
Desmethylimipramine	1.44	82.6 ± 0.9	(5)	0.0025
Zimelidine	0.15	81.0 ± 0.6	(5)	–
Paroxetine	0.043	84.4 ± 1.0	(5)	0.37
Citalopram	0.062	80.2 ± 1.2	(5)	–
5-HT	4.8	–		33.0
(−)-NA	> 100	–		3.2

Ganglia were incubated with [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) 1 × 10^{-8} M, or (−)-[^{3}H]-noradrenaline, 1 × 10^{-8} M. IC_{50} values were determined by direct fit of the logistic curve to the concentration-inhibition curve for each compound; estimated standard errors were less than 10%. Values for the maximum inhibition of [^{3}H]-5-HT uptake were determined in the presence of chlorimipramine, 1 × 10^{-5} M, or desmethylimipramine, zimelidine, paroxetine, or citalopram at 1 × 10^{-4} M, all in the same experiment.

being non-significant ($P > 0.05$, analysis of variance). Values for IC_{50} were determined from the fitted curves; supra-maximal concentrations of the inhibitors all produced approximately similar inhibition of ganglionic [^{3}H]-5-HT uptake (Table 2).

Metoclopramide caused significant inhibition ($P < 0.05$) of the accumulation of radioactivity by ganglia incubated with [^{3}H]-5-HT, 1×10^{-8} M, only at concentrations of 3×10^{-5} M or greater.

Kinetics of the uptake of [^{3}H]-5-HT by the rat superior cervical ganglion

A plot of the rate of total 5-HT uptake against [^{3}H]-5-HT concentration over the range 1×10^{-8} -1×10^{-5} M, shows some deviation from linearity, but no appreciable saturation (Figure 3). It is possible that this uptake may have comprised more than one component, since it was not completely blocked by any of the inhibitory procedures tested (see Tables 1 and 2). Therefore an analysis was made of the kinetics of those elements of [^{3}H]-5-HT uptake sensitive to inhibition by ouabain, low temperature or a supra-maximal concentration of a 5-HT uptake inhibitor. Figure 3 shows the chlorimipramine-sensitive component of total 5-HT uptake determined by subtraction of the element resistant to chlorimipramine, 1×10^{-5} M. Chlorimipramine-sensitive uptake exhibited marked saturation; kinetic constants were determined by direct fit of a hyperbola to the data. The ouabain-sensitive and cold-sensitive components of total 5-HT uptake were determined similarly. The derived kinetic constants are shown in Table 3.

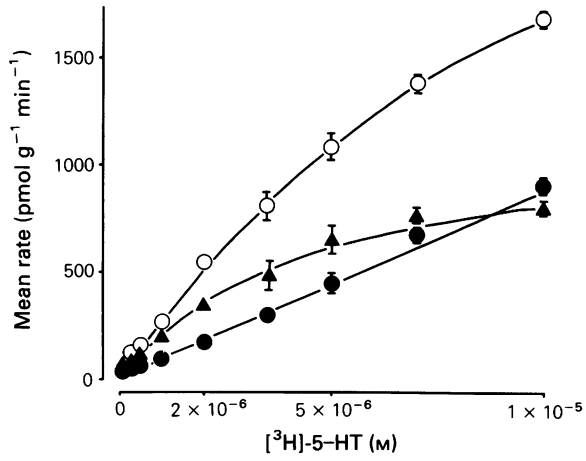

Figure 3 Kinetics of the accumulation of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) by rat superior cervical ganglion. Points show experimentally determined total (○), and chlorimipramine-resistant (●) accumulation. Chlorimipramine-sensitive uptake (▲) was calculated from individual values of the total accumulation by subtraction of the appropriate mean chlorimipramine-resistant rate. The latter was determined in separate ganglia incubated with chorimipramine 1×10^{-5} M. Points are means, with vertical lines indicating s.e.mean, of results from at least 4 individual ganglia. The straight line and the hyperbola were each fitted using a computer program.

The effects of uptake inhibition on the antagonist potency of metoclopramide.

The 5-HT uptake inhibitor paroxetine was used in studies on the possible effects of uptake inhibition on 5-HT-induced depolarization of the VN and SCG. This compound was chosen in preference to the other uptake inhibitors since, in preliminary studies, it appeared the least prone to antagonize 5-HT-induced responses at concentrations producing significant inhibition of 5-HT uptake (results not shown). The effects of uptake inhibitors on 5-HT-induced depolarization were measured after a minimum of 60 min exposure to the drug.

On the VN, paroxetine, $3 \times 10^{-7} - 1 \times 10^{-6}$ M, had no significant effect on 5-HT-induced depolarization responses, although at 3×10^{-6} M it caused attenuation. The principal effect was a reduction in the maximum response. On the SCG, paroxetine, 1×10^{-6} M, caused a leftward shift of the 5-HT concen-

Table 3 Kinetic constants for [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake in rat superior cervical ganglion

	[^{3}H]-5-HT uptake	
Uptake component	K_M (μM)	V_{max} (pmol g^{-1} min^{-1})
Chlorimipramine-sensitive	4.7	1187
Ouabain-sensitive	3.9	926
Cold-sensitive	6.7	1848

Values were determined by direct fit of a hyperbola to the calculated chlorimipramine-sensitive, ouabain-sensitive, or cold-sensitive components of total 5-HT uptake. Standard errors were approximately 5%, but these do not include the error induced by calculating the inhibition-sensitive components of total 5-HT uptake.

tration-depolarization curve with no change in the maximum response; in the presence of paroxetine, 1×10^{-6} M, the mean EC_{50} ($\pm$ s.e.mean) for 5-HT was $2.7 \pm 0.2 \times 10^{-6}$ M ($n = 40$) compared to $1.4 \pm 0.1 \times 10^{-5}$ M ($n = 36$) in the absence of the uptake inhibitor. However, at a concentration of 3×10^{-6} M, which is only slightly higher than that found to have this potentiating effect, paroxetine inhibited 5-HT-induced depolarization. As on the VN the principal effect was a reduction in the maximum response. Paroxetine, 3×10^{-7} M, had no significant effect on 5-HT-induced depolarization of the SCG.

The NA-uptake inhibitor desmethylimipramine, 1×10^{-7} M, had no significant effect on 5-HT-induced depolarization of the VN or SCG (results not shown). In the SCG, this concentration of desmethylimipramine caused inhibition of (−)-[^{3}H]-NA but not of [^{3}H]-5-HT accumulation.

The effects of metoclopramide against 5-HT-induced depolarization were examined in VN and SCG preparations exposed to paroxetine, 1×10^{-6} M. On the VN the uptake inhibitor did not modify the potency of metoclopramide (Figure 2). On the SCG, in the presence of paroxetine, metoclopramide, 1×10^{-6}–1×10^{-4} M, produced parallel rightward shifts of the 5-HT concentration-response curve. However, the gradient of the Schild plot was 0.93 ($\pm$ 0.04), compared to the value of 0.82 ($\pm$ 0.12) obtained in the absence of the uptake inhibitor. Neither value was significantly different from unity ($P > 0.05$) (Figure 2). The corresponding pK_B values were 6.25 ($\pm$ 0.03, $n = 20$) and 5.74 ($\pm$ 0.07, $n = 16$), respectively. The apparent potency of metoclopramide as an antagonist of phenylbiguanide-in-

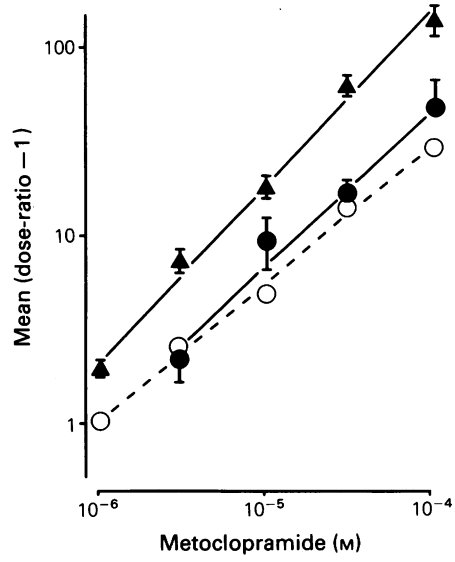

Figure 4 Use of Furchgott's (1972) model to predict the effect of 5-hydroxytryptamine (5-HT) uptake on the apparent 5-HT antagonist potency of metoclopramide on the rat superior cervical ganglion (SCG). Filled symbols and solid lines represent observed results; each symbol indicates the mean, with vertical lines representing s.e.mean, of single determinations on 4 separate tissues. (▲) Data recorded from ganglia exposed to paroxetine, 1×10^{-6} M. (●) Results obtained in the absence of the 5-HT uptake inhibitor. Furchgott's model was used to predict the influence of chlorimipramine-sensitive 5-HT uptake by the SCG, on the data recorded in the presence of paroxetine. These calculated results are indicated by (O - - - O).

Table 4 The observed and predicted effects of 5-hydroxytryptamine (5-HT) uptake on the apparent 5-HT-antagonist potency of metoclopramide on the rat superior cervical ganglion (SCG)

Source of result	Apparent 5-HT-antagonist potency of metoclopramide on rat SCG $pK_B \pm s.e.$	(n)	*slope* $\pm$ *s.e.*
A Observed in presence of paroxetine 1×10^{-6}M	6.25 ± 0.03	(20)	0.93 ± 0.04
B_1 Calculated from A for CMI-sensitive uptake	5.73		0.73
B_2 Calculated from A for cold-sensitive uptake	5.77		0.71
B_3 Calculated from A for ouabain-sensitive uptake	5.80		0.76
C Observed in absence of paroxetine	5.74 ± 0.07	(16)	0.82 ± 0.12

A and C show the data observed in the presence and absence of paroxetine, 1×10^{-6} M, respectively. The slope values are the gradients of plots of log (dose-ratio − 1) against log (antagonist concentration). The predicted effects on the results shown in A, of chlorimipramine (CMI)-sensitive, cold-sensitive, and ouabain-sensitive ganglionic 5-HT uptake, were estimated using Furchgott's (1972) model (see text and Figure 4).

duced depolarization of the SCG, was unaffected by paroxetine (results not shown).

The selective NA-uptake inhibitor desmethylimipramine, 1×10^{-7} M, did not alter the apparent potency of metoclopramide as an antagonist of depolarization responses induced by 5-HT on the SCG: the Schild plot slope and pK_B value were 0.83 (± 0.09) and 5.76 (± 0.05, $n = 16$), respectively.

Comparison of the experimental results with those predicted by Furchgott's (1972) model

Furchgott (1972) devised the following equation to simulate the influence of saturable agonist uptake on the relationship between the concentration of agonist in the external medium ([Aa]) and that at equilibrium with the receptors ([Ab]), in the presence of various concentrations of a reversible competitive antagonist ([B]):

$$[Aa] = [Ab]_o(1 + [B]/K_B)\left[1 + \frac{\frac{V_{max}}{kK_M}}{1 + \frac{[Ab]_o(1 + [B]/K_B)}{K_M}}\right]$$

where k is a constant for movement of the agonist into the region of the receptors. We have used this relationship to predict the effect of 5-HT uptake on the 5-HT antagonist activity of metoclopramide on the SCG, using the maximum rate (V_{max}) and half-maximal rate constant (K_M) values for uptake, determined in the present study. It was assumed that the 5-HT uptake system was inactive in the presence of paroxetine, 1×10^{-6} M.

Figure 4 and Table 4 reveal good agreement between the predicted and observed data.

Discussion

Metoclopramide caused parallel rightward displacements of the 5-HT concentration-depolarization curves on both the rat VN and SCG. The pK_B values calculated from the effects of this antagonist measured in the absence of a 5-HT uptake inhibitor were 6.60 (± 0.04) and 5.74 (± 0.07), respectively. These differed sufficiently to suggest that the 5-HT receptors that mediate depolarization of the rat VN may not be identical to those on the SCG. We have previously shown that depolarization induced by even high concentrations of 5-HT applied to the VN and SCG both in the presence and absence of metoclopramide, 1×10^{-4} M, was resistant to blockade by a variety of non-5-HT antagonists (see Ireland *et al.*, 1982; Fortune *et al.*, 1983). This makes it unlikely that the relatively low potency of metoclopramide as a 5-HT antagonist on the SCG was due to activation of non-5-HT receptors by elevated concentrations of the agonist.

In this paper, we have examined an alternative suggestion prompted by the work of Langer & Trendelenburg (1969), who proposed that the potency of a competitive antagonist may be underestimated if the agonist against which it is tested is also the substrate for a saturable uptake system within the test tissue.

It was found that both whole SCG preparations and VN segments accumulated tritium during incubation with a wide range of [^{3}H]-5-HT concentrations. With the VN, no evidence was obtained to suggest that this accumulation was saturable. In contrast, with the SCG, the elements of total [^{3}H]-5-HT uptake sensitive to inhibition by chlorimipramine, ouabain, or low temperature appeared saturable: each could be adequately described by a single hyperbola. The possibility that these apparently saturable elements comprised heterogeneous transport systems was not investigated. The value of the half-maximal rate constant (K_M) for saturable ganglionic [^{3}H]-5-HT uptake was close to the threshold concentration of 5-HT required to depolarize the tissue. However, it is unlikely that the apparent saturation of uptake was due to depolarization, since phenylbiguanide did not affect the accumulation of [^{3}H]-5-HT by the SCG, even though it mimics the depolarizing activity of 5-HT in this preparation (Fortune *et al.*, 1983).

Results obtained with a range of uptake inhibitors against the accumulation of radiolabel by ganglia incubated with a low concentration of [^{3}H]-5-HT, suggested a similarity between 5-HT uptake systems in the rat SCG and brain (see Ross, 1982). The former appeared distinct from (−)-[^{3}H]-NA accumulation (Table 2). 5-HT did inhibit (−)-[^{3}H]-NA accumulation, although the concentrations required were greater than those needed to block [^{3}H]-5-HT uptake.

In the present study, no attempt was made to compare the location of the 5-HT uptake processes within the SCG with the sites at which the agonist acted to cause depolarization. It is also unclear to what extent the apparently saturable accumulation of [^{3}H]-5-HT by this tissue indicated a net uptake of 5-HT, as opposed to exchange of radiolabelled for endogenous material (see Cerrito & Raiteri, 1979). The latter is possible since the 5-HT-synthetic enzyme tryptophan hydroxylase, and cells specifically containing 5-HT-like immunoreactivity have been found in the rat SCG (Saavedra & Luizzi, 1978; Verhofstadt *et al.*, 1981). However, evidence was obtained that 5-HT uptake can influence the effect of 5-HT on the rat isolated SCG preparation. Thus, when 5-HT-induced depolarization responses were recorded from SCG preparations exposed to the 5-HT uptake inhibitor paroxetine, 1×10^{-6} M, it was found that the 5-HT

concentration-depolarization curve was shifted to the left of the control, and that the apparent potency of metoclopramide as a 5-HT antagonist was increased. It is likely that this effect of paroxetine was due to inhibition of 5-HT uptake *per se*. Thus, paroxetine, 1×10^{-6} M, did not affect the potency of metoclopramide as an antagonist of the depolarization induced by the 5-HT-mimetic phenylbiguanide. This is consistent with the low affinity of phenylbiguanide for the 5-HT uptake process. Further, desmethylimipramine, at a concentration (1×10^{-7} M) that selectively blocks ganglionic NA but not 5-HT uptake, did not modify the effects of 5-HT or metoclopramide on the SCG. Finally, on the VN, paroxetine, 1×10^{-6} M, did not change the apparent potency of either metoclopramide or 5-HT.

The experimental observations of the effects of paroxetine on the 5-HT antagonist potency of metoclopramide on the SCG were compared with those predicted by Furchgott's (1972) model. The form of the model employed assumes that the antagonist has no effect on agonist uptake. This seemed appropriate since metoclopramide was found to reduce [^{3}H]-5-HT uptake only at a concentration at least 30 fold in excess of those required to antagonize 5-HT-induced depolarization. The model requires values for the V_{max} and K_M of the uptake system studied. In the present experiments, these depended on whether the cold-sensitive, ouabain-sensitive or chlorimipramine-sensitive component of total 5-HT uptake was analysed. However, the use of these different estimates did not cause material change to the predicted influence of 5-HT uptake on the apparent potency of metoclopramide as a 5-HT antagonist (see Table 4). This was characterized by a small rightward shift and decline in slope of the plot of log dose-ratio − 1 against log antagonist concentration. In all cases, the predicted plots deviated slightly from linearity (Figure 4). There was close agreement between the predicted and observed data for antagonism by metoclopramide of 5-HT-induced depolarization of the SCG in the absence of an uptake inhibitor.

In conclusion, the results are consistent with the suggestion that the saturable uptake of [^{3}H]-5-HT by the rat SCG reduced the apparent potency of metoclopramide as a 5-HT antagonist on this tissue. In contrast to the SCG, the VN was shown not to accumulate [^{3}H]-5-HT via a saturable mechanism. Therefore, it is likely that the observed difference in the potency of metoclopramide as a 5-HT antagonist on these two tissues was due to the influence of 5-HT uptake, rather than the existence of sub-types of 5-HT receptors. The influence of 5-HT uptake on the apparent potency of other 5-HT antagonists on the rat SCG has yet to be tested. It is interesting to note that Round & Wallis (1986) found almost identical pA_2 values for the antagonism by ICS 205-930 of 5-HT-induced depolarization of the rabbit vagal and SCG neurones. These results were obtained in the absence of a 5-HT uptake inhibitor since in the rabbit SCG, the effects of 5-HT do not seem to be influenced by 5-HT uptake (Round & Wallis, 1986).

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